

# FIELD EXPERIMENTS ON THE ASSOCIATION OF DECAPOD CRUSTACEANS WITH SEA ANEMONES, *ANEMONIA VIRIDIS* (FORSSKÅL, 1775)

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We have undertaken for the first time removal and introduction field experiments with both symbiotic (taken from anemones) and anemone-naïve decapods (taken from somewhere else) and tested defence reactions of putative host anemones. Our findings indicate that decapods go through an individual habituation phase before they become symbionts. Once habituated, they are protected from neighbouring anemones as well.

**Key words:** symbiosis, field experiments, sea anemones, decapods

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Po prvi puta smo proveli terenske pokuse sa simbiotskim dekapodnim rakovima (uzetim s vlasulja) i onima koji nisu u kontaktu s vlasuljama (uzeti s drugih mjesta) te smo testirali obrambene reakcije vlasulja-domaćina. Naši rezultati pokazuju da dekapodni rakovi prolaze individualnu fazu navikavanja prije nego što postanu simbioti. Nakon toga budu zaštićeni i od susjednih vlasulja.

**Ključne riječi:** simbioza, terenski pokusi, moruzgve i vlasulje, dekapodni rakovi

## INTRODUCTION

Decapod crustaceans living as symbionts, commensals or ectoparasites on sea anemones and other cnidarian hosts is a common phenomenon observed for numerous species of decapods as well as cnidarians (WEINBAUER *et al.*, 1982; WIRTZ & DIESEL, 1983; ROSS, 1983; FAUTIN *et al.*, 1995). In many aspects this relationship is similar to teleost-cnidarian associations, e.g. those of clown fish, *Amphiprion* and anemones (e.g., FRICKE, 1974, 1975; SCHLICHTER, 1976; MIYAGAWA & HIDAKA, 1980; BROOKS & MARISCAL, 1984; MEBS, 1994). In both cases the symbionts have to handle the cnidocyst defence shield of their hosts.

In addition to species lists (WIRTZ, 1997; PATZNER, 2004), various aspects of the behavioural ecology of this classical example for mutualism have been studied, e.g. host selection (BROOKS & RITTSCHOF, 1995; KHAN *et al.*, 2003; CALADO *et al.*, 2007), host use patterns (BAEZA & THIEL, 2003), protection (MIHALIK & BROOKS, 1997), territoriality (BAEZA *et al.* 2002), social structure and behaviour (WIRTZ & DIESEL, 1983; THIEL & BAEZA, 2001; KHAN *et al.*, 2004) and male-female associations (DIESEL, 1988). WIRTZ (1997) has pointed out that only a few species form lasting associations with anemones, e.g. partner shrimps (*Periclimenes* spp.) and the majid *Inachus phalangium*, while other species found on anemones are short term visitors or facultative symbionts. Recently, CALADO *et al.* (2007) inferred from their choice experiments that some decapods, especially those observed near the anemone's body but not in contact with the tentacles, might be accidental visitors or even use the anemone shortly as a hideout when threatened.

Looking at all these studies, it is remarkable that the degree of protection against the anemones' cnidocysts of anemone visiting decapods has never been tested in field experiments. In the present study we could do this by virtue of an advantageous study area, i.e. an infralittoral *Anemonia viridis* bed housing a multi-species decapod assemblage on a comparatively small area. In Saline Bay, at the vicinity of Rovinj (Croatia, Northern Adriatic Sea), one finds a large anemone meadow of ca. 50 m<sup>2</sup> with the anemones covering more than 50% of the ground composed of rocks, stones and small sediment spots at the level of the upper infralittoral, directly underneath a mediolittoral *Fucus virsoides* seaweed community. According to the UNEP classification of Mediterranean marine communities (1996), such an association is referred to as *Anemonia viridis* facies. *Anemonia viridis* is also better known under an old name, *Anemonia sulcata*.

Herein we recorded the decapod visitors of this particular location rather than collecting data over a larger area as has been done in other studies (WIRTZ, 1997; PATZNER, 2004). We examined whether the species were sitting directly in the anemone's tentacles. Those were assumed to be habituated to their hosts as facultative symbionts *sensu* WIRTZ (1997), rather than accidental visitors. To test for habituation, we conducted »remove and introduce« experiments, i.e. we placed anemone-naïve as well as symbiotic decapods on the anemones and checked if there was a reaction of the tentacles or not. GIESE *et al.* (1996) had previously used this technique in an aquarium experiment, but field test had never been undertaken. We show here that decapods living in the *Anemonia viridis* meadow at Saline Bay are indeed individually habituated to their hosts. Anemone-naïve decapods of the same species and/or of different species taken from the surroundings are attacked and therefore not habituated. Furthermore there is good indication for the state of adaptation being population or even species specific with respect to the host anemones.

## ANIMALS AND METHODS

### Listing the symbionts

We made our observations during more than 25 SCUBA and snorkel trips from January 2003 to August 2008, mostly by night, when the studied decapods were active. We chose this time as the majority of decapods then had left their hiding

places and were present in the anemone meadow. Only several specimens were removed from the meadow in order to enable us to properly identify the species without excessive disturbance. Canon IXUS 400 and 850 underwater cameras were used for photographic documentation of the species occurring in the meadow. Only individuals that had direct contact with the anemone's tentacles and provoked no defence reaction from the anemones were included in our species list. In addition, a list of non-anemone inhabiting decapods from the surroundings was also compiled.

### General design of the »remove and introduce« experiments

During a night snorkelling trip at Saline Bay we observed that an anemone inhabiting *Pilumnus* crab moved actively from one anemone to another within the meadow without being harmed, and thus obviously was adapted to more than only one anemone. Conversely a crab that actually had no contact with an anemone provoked a persuasive reaction when exposed to anemone tentacles: Anemone tentacles moved in a manner typical of a prey-capture or defensive response and were pasted firmly on the crab. At this, the crab was not seriously damaged, as it could actively leave the anemone. Based on this observation, we tested numerous individuals belonging to various decapod taxa through a set of experiments using the defence reaction of the anemones as a field assay for the decapod's state of habituation.

Decapods were taken from their original location either on an anemone or the surrounding area and either transported in a vial or by hand to the test anemone. These were then dropped onto the anemone from 10 to 20 cm above. Since *Anemonia viridis* has rather long tentacles, the first crab-anemone contact was with the tentacles, and not parts of the body column or the mouth field. Handling of the decapods by hand theoretically could alter the state of adaptation. However, this would provoke unexpected reactions, i.e., a naïve crab would not be stung. We did not observe any of such reactions.

The observed reactions from the crabs dropped onto anemones were generally very clear, i.e. the decapods were either stung by tentacles or they were not. Only 3 of 84 tests did not allow an unambiguous statement. The 2 types of reactions we observed can be characterized as follows:

1. At the introduction of the decapod, the anemone's tentacles immediately moved actively to the intruder and stuck to its body. In reaction the decapod could not move freely, and either secluded itself or tried to flee. While moving, it was clearly visible that the tentacles were still glued to the carapace or body appendages of the decapod. For decapods not moving we used an additional »push« test, i.e. we shifted the decapod away from its original place to see if tentacles were glued to the body or not. Decapods provoking these types of reaction was rated as »non-habituated«. The decapods themselves reacted in quite different ways to the anemone's attacks: Some did not move at all though tentacles attacked them, some immediately sought to withdraw, and some others, especially the very fast swimming forms, such as *Galathea* sp., rushed away from the tentacles in a flash upon contact.

2. At the introduction of the decapod, the anemone's tentacles did not attack, they did not stick to the decapod's body, and the decapod did not seek to flee nor

was it handicapped in its movements. The push test showed that the decapod could be freely moved among the tentacles. A decapod provoking a reaction of this type was rated as »habituated«.

### The specific design of the experiments

Theoretically, the adaptation of a decapod to anemones could be individually acquired during anemone contact thus being a kind of habituation, or represent a feature of an individual or a species present already before the first contact with an anemone has occurred. Furthermore the habituation could work for only a single anemone, but it could also work for all the individuals belonging to a clone, population, species, or even related species. Our approach focuses on decapods that were found on anemones (all of them were taken from *Anemonia viridis* individuals) as well as presumably anemone-naïve decapods that were found somewhere else in the neighbourhood of the test anemones. Transfer of decapods from one anemone to another studies the specificity of the adaptation, while transfer of decapods not sitting in anemones onto anemones studies if there is a pre-adaptation or not. The area from which test decapods were taken covers approximately 40.000 m<sup>2</sup> (4 ha) including the anemone meadow.

Experiment I: If decapods are habituated to only one individual anemone they should be attacked when transferred to another anemone within the meadow. To test this, we took 10 decapods from the meadow (3 *Inachus* sp., 5 *Pilumnus* sp. and 2 *Xantho* sp.), and dropped them onto neighbouring anemones within the meadow.

Experiment II: To test if the habituation is acquired or if there is some pre-adaptation we took 14 individuals of the same taxa as in experiment I not sitting on anemones (9 *Pilumnus* sp., 3 *Xantho* sp., 2 *Inachus* sp.), and introduced them into the meadow.

Experiment III: Decapods of other species could also be pre-adapted. Hence, we took 24 anemone-naïve decapods from the surroundings (9 *Galathea* sp., 6 *Ilia* nucleus, 3 *Pisidia longicornis*, 4 *Porcellana platycheles*, 2 *Carcinus aestuarii*) and dropped them.

Experiment IV: Habituation acquired on an anemone in the neighbourhood of the meadow might work or not work in the anemone meadow. To test this, we removed 12 anemone symbionts (7 *Inachus* sp., 3 *Macropodia* sp., 2 *Periclimenes amethysteus*) from their hosts in the study area (i.e., between 50 and 200m away from the meadow) and introduced them into the meadow. For this experiment we only used decapods sitting within the tentacle area of their hosts and having contact with them without being attacked.

Experiment V: As a cross check of experiment IV, we took 14 symbionts (6 *Inachus* sp., 6 *Pilumnus* sp., 2 *Xantho* sp.) from the meadow, and dropped them on other anemones in the test area (i.e., between 50 and 200 m away from the meadow).

### Analysis of the results

All the dropping events were classified into one of the 2 categories described above. Afterwards, results were analysed in two ways: (1) A taxon-specific interpretation allowed to detect tendencies on the adaptive state of the individuals of each

**Tab. 1:** List of species found in the *Anemonia* meadow

Caridea	Palaemonidae	<i>Periclimenes amethysteus</i> (Risso, 1827)
Anomura	Paguridae	<i>Clibanarius erythropus</i> (Latreille, 1818) <sup>1)</sup>
Brachyura	Majidae	<i>Inachus communissimus</i> Rizza, 1839 <sup>2)</sup>
		<i>Inachus leptochirus</i> Leach, 1817 <sup>1) 2)</sup>
		<i>Inachus phalangium</i> (Fabricius, 1775) <sup>2)</sup> (Fig. 1B)
		<i>Macropodia longirostris</i> (Fabricius, 1775)
		<i>Macropodia rostrata</i> (L., 1761)
		<i>Herbstia condyliata</i> (Fabricius, 1787) <sup>1)</sup>
		<i>Maja squinado</i> (Herbst, 1788)
	Pilumnidae	<i>Pilumnus hirtellus</i> (Linnaeus, 1761)
		<i>Pilumnus villosissimus</i> (Rafinesque, 1814) (Fig. 1D, F)
	Xanthidae	<i>Xantho poressa</i> (Olivi, 1792)
		<i>Xantho pilipes</i> A. Milne Edwards, 1867
		<i>Eriphia verrucosa</i> (Forskål, 1775) (Fig. 1C)
	Grapsidae	<i>Pachygrapsus marmoratus</i> (Fabricius, 1787) <sup>1)</sup>

**Tab. 2:** List of species found around the *Anemonia* meadow, but not inside

Penaeidea	Penaeidae	<i>Sicyonia carinata</i> (Brünnich, 1768) <sup>1)</sup>
Caridea	Hippolytidae	<i>Lysmata seticaudata</i> (Risso, 1816)
		<i>Thoralus cranchii</i> (Leach, 1817)
	Palaemonidae	<i>Palaemon elegans</i> Rathke, 1837
		<i>Palaemon serratus</i> (Pennant, 1777)
Anomura	Galatheidae	<i>Galathea squamifera</i> Leach, 1814
		<i>Galathea strigosa</i> (L., 1767)
	Porcellanidae	<i>Pisidia longicornis</i> (L., 1767)
		<i>Porcellana platycheles</i> (Pennant, 1777)
Brachyura	Leucosiidae	<i>Ilia nucleus</i> (L., 1758) <sup>1)</sup> (Fig. 1E)
	Majidae	<i>Pisa armata</i> (Latreille, 1803)
	Portunidae	<i>Carcinus aestuarii</i> Czerniavski, 1884 <sup>1)</sup>

taxon studied (Tab. 3), while (2) pooled data were large enough to analyze them with the chi-square test, the null hypothesis being a random reaction of the anemones on which a decapod was introduced ( $n$ ,  $\chi^2$  and  $p$  values are given in Tab. 4).

### Reactions on tentacle's attacks

Many of the bigger individuals, especially the robust crabs like *Ilia*, *Xantho* and *Pilumnus*, were able to disengage themselves from the sticking tentacles. The more delicate and smaller species had difficulties escaping and were removed by hand

**Tab. 3:** Summary of results for all decapods used in the experiments. *Inachus* - *Xantho*: taxa that occur as *Anemonia* symbionts; *Carcinus* - *Porcellana*: taxa not observed as symbionts.

Taxon	taken from anemone	taken from other locations	attacked by target anemone	not attacked by target anemone
<i>Inachus</i> sp.	16		1	15
		2	2	
<i>Macropodia</i> sp.	3			3
<i>Periclimenes amethysteus</i>	2			2
<i>Pilumnus</i> sp.	11		1	10
		8	8	
<i>Xantho</i> sp.	4			4
		3	3	
<i>Carcinus aestuarii</i>		2	2	
<i>Galathea</i> sp.		9	8	1
<i>Herbstia condyliata</i>		1	1	
<i>Ilia nucleus</i>		6	6	
<i>Pisidia longicornis</i>		3	3	
<i>Porcellana platycheles</i>		4	4	

**Tab. 4:** Summary of experiments I-V, plus combined results I&IV&V and II&III

Type of experiment	Number of individuals n	Reaction 1	Reaction 2	Habituated?	$\chi^2$	p
I: Inhabitant of an anemone transferred to another anemone in the meadow	10	0	10	yes	6.667	0.00982
II: Anemone-naïve decapod of a species that can be found in the meadow	14	14	0	no	9.333	0.00225
III: Anemone-naïve decapod of a species that can not be found in the meadow	24	23	1	no	12.765	0.00035
IV: Habituated decapod removed from neighbouring anemone and then introduced into the meadow	12	1	11	yes	5.042	0.02474
V: Habituated decapod removed from meadow and introduced to anemone in the neighbourhood	14	1	13	yes	6.8069	0.00908
I, & IV & V: All individuals transferred from one anemone to another	36	2	34	yes	17.723	0.00002
II & III: All anemone-naïve decapods dropped on anemone	38	37	1	no	21.986	0.000003

from the anemones. We took care that all the experimental decapods from outside the meadow were put somewhere else after the test was done. They were apparently all in good shape. It seems that their cuticle is thick enough to avoid penetration by discharged nematocysts and intoxication. Thus the tentacles sticking to the decapods body is the main effect of the anemone's defence reaction.

## RESULTS

### Observed decapod species

Tab. 1 summarizes decapod species that were observed in the *Anemonia viridis* meadow (Fig. 1a), contacting the anemones without being stung or provoking any other defence reaction. These species were found in the neighbourhood of the meadow as well. Species labelled with <sup>1)</sup> were seen only once or very rarely, while the others were common and observed at almost every visit. Those labelled with <sup>2)</sup>, the 3 *Inachus*, 2 *Macropodia*, 2 *Pilumnus* and 2 *Xantho* species, were identified in the lab using only a few individuals. The majority, however, were only determined to the genus level in the field to avoid removal of large amounts of decapods from their habitat.

Only *Periclimenes amethysteus* and *Inachus* spp. were observed in another neighbouring anemone species, i.e. *Aiptasia mutabilis* (Gravenhorst, 1831). Here, we occasionally also found *Periclimenes aegylios* (Grippa and Udekem d'Acoz, 1996), a second Mediterranean partner shrimp species not observed in *Anemonia viridis* in Saline Bay until present. Most of the *Xantho*, *Pilumnus* and *Eriphia* we observed in the meadow had a hideout (small cave or burrow) they used for escape when seriously disturbed directly next to the anemone in which they were found.

There are, however, other common decapods often present in close vicinity of the meadow, but never inside. These are listed in Tab. 2. Of these, species labelled with <sup>1)</sup> were seen on sediment bottom below the *Anemonia* meadow, while the others occurred at the same depth on the rocky slope of the upper infralittoral, where the meadow is located.

### Removal and introduction experiments

Results of our experiments are summarized in Tabs 3 and 4. Of these, Tab. 3 refers to the different taxa studied and shows individual reactions to the exposure. Tab. 4 summarizes the results with respect to the origin of the decapods and their state of habituation.

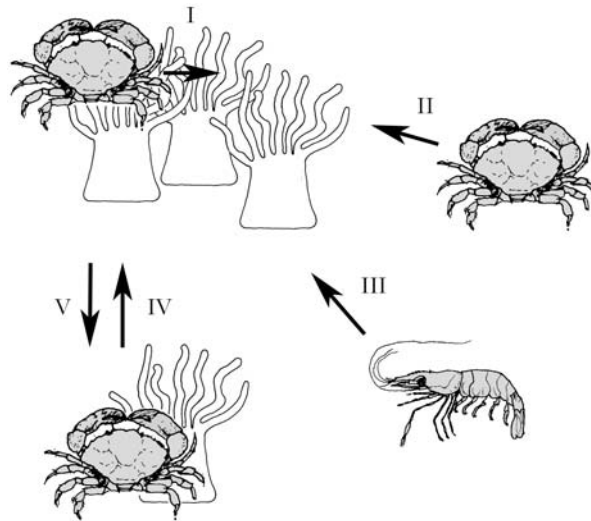
In Tab. 3, the vast majority of decapod symbionts taken from anemones (such as *Inachus*, *Pilumnus* and *Xantho*) provoked no defence reaction when transferred to other *Anemonia viridis* individuals in the neighbourhood. Individuals of the same species taken from elsewhere were stung when dropped on an anemone. Their reaction thus depends on their origin, and habituation to the host anemones is thus an individually acquired rather than a species-specific feature of the decapods. We could not make a cross-check for *Macropodia* sp. and *Periclimenes amethysteus*, as we found them only as symbionts and could test only a few individuals.



**Fig. 1.** A. *Anemonia viridis* meadow. B-D. Host-adapted decapods in their anemones. B. *Inachus phalangium*. C *Eriphia verrucosa*. D. *Pilumnus villosissimus*. E, F. Anemone-naïve decapods attacked after their introduction. E *Ilia nucleus*, a species never found associated with anemones in the studied bay, heavily attacked by *Anemonia viridis* after introduction; note tentacles glued onto crab at various parts of the body. F Anemone-naïve *Pilumnus villosissimus*, hence an individual belonging to a species that is facultatively symbiotic, after introduction in reverse gear seeking for its way out of the anemonias after attack; note numerous tentacles glued onto the anterior body part of the crab.

For the tested species not found on anemones in Saline Bay, it becomes clear that all of them lack pre-adaptation, as almost all the tested anemone-naïve individuals provoked a defence reaction. However, the number of individuals from most of the





**Fig. 2.** Schematic illustration of the »remove and introduce« experiments made. Upper left: *Anemonia* meadow, lower left: single *Anemonia* located at a distance of between ca. 30 and 200m from the meadow. I: Decapod associated with an *Anemone* is transferred to other anemones within the meadow. II: Anemone-naïve decapod belonging to a facultatively symbiotic species is introduced into the meadow. III: Anemone-naïve decapod belonging to a species never found associated with anemones is introduced into the meadow. IV: Anemone-adapted decapod is removed from its primary host, and introduced into the meadow. V: Anemone-adapted decapod is removed from the meadow, and introduced into a secondary anemone in the vicinity of the meadow.

tested taxa are low, and therefore the conclusions drawn above are no more than tendencies. Pooling of the data, however, allows a more detailed interpretation.

Tab. 4 summarizes our combined results from experiments I-V. Individuals of different taxa are pooled with respect to their origin and the target anemones on which they were dropped. In addition the total amount of the adapted and non-adapted individuals were tested. This allows the number of individuals in each category to be high enough for analysis using the chi-square test ( $n$ ,  $\chi^2$  and  $p$  values are given in Tab. 4).

Data shown in Tab. 4 (see also Fig. 2) support and refine the conclusions drawn from Tab. 3. They also show, for the first time in field experiments at an acceptable significance level, that a decapod habituated to one individual *Anemonia viridis* is habituated to others (experiment I & IV & V). Furthermore anemone-naïve decapods (both of the same species, as well as others) are not, and are stung by anemones when introduced (experiment II & III).

To summarize, our experiments address particular questions that lead to the following results:

Experiment I. Are the inhabitants of the meadow habituated to only one anemone, or to any anemone within the meadow? To answer this question, 10 adapted decapods were moved from one anemone to another within the meadow, and none

of them were attacked or intoxicated by the anemone. Habituation thus works all over the meadow, not only in a single anemone individual.

Experiment II. Are conspecifics living nearby the anemones attacked when put into the meadow? The 12 tested anemone-naïve decapods were all attacked. Hence, the habituation is an acquired, individual feature, not a population one. No individual showed signs of any pre-adaptation.

Experiment III. How do anemones react to anemone-naïve decapods belonging to species otherwise not found in the meadow when dropped? 23 of 24 decapods were attacked, and hence were assumed to not be pre-adapted.

Experiment IV. Are decapods living in nearby *Anemonia viridis* (ca. 30–200 m) protected when introduced into the meadow? 11 of 12 induced reaction of type 2. Hence these decapods were habituated before contact with anemones in the meadow.

Experiment V. Are decapods living in the *Anemonia* meadow protected when introduced to anemones of the same species planted in the neighbourhood (ca. 30–200 m)? 13 of 14 induced a reaction of type 2. Hence these decapods were pre-adapted to contact with other *Anemonia viridis*. Experiments IV and V show that habituation, once acquired, works on other anemones of the same species planted in the neighbourhood.

## DISCUSSION

### Species lists

Along with *Periclimenes amethysteus* and *Inachus phalangium*, our list includes the classical symbionts of anemones in the Mediterranean (WIRTZ & DIESEL, 1983; PATZNER, 2004). The other constituents are common inhabitants of the mediolitoral and/or upper infralitoral of Saline Bay and were found at locations around the meadow as well. In general our observations correspond well with the expected habitat needs of the species, as summarized in ZARIQUIEY ALVAREZ (1968). Except for *Periclimenes amethysteus*, and eventually *Inachus phalangium*, they are also found outside the meadow and hence are facultative symbionts. If one compares our results with the list given in CALADO *et al.* (2007), one can see that there are many regional differences. *Xantho incisus* and *Eriphia verrucosa*, species common on the anemones of Saline Bay, were not found on anemones in Portugal, though it's within their geographical range. This seems to account also for other anemones, e.g. *Telmatactis cricoides* (WIRTZ, 1997). In this location, *Lysmata seticaudata* is a common anemone symbiont, but not in the anemones in Saline Bay, though *Lysmata* is present in the habitat. It is suggested that presence or absence of a species as a symbiont widely depends on the habitat conditions. The large area *Anemonia viridis* facies at Saline Bay seem to allow numerous upper infralittoral decapods to become facultative symbionts.

### Acquired immunity

The main results of our field experiments are that numerous decapod symbionts are not only habituated to a single anemone, but also to other anemones of the

same species in the neighbourhood. Also anemone-naïve decapods do not possess an innate adaptation to the anemones, since they are stung. As has been indicated earlier in aquarium experiments made by LEVINE & BLANCHARD (1980), CRAWFORD (1992) and GIESE *et al.* (1996), they obviously go through a habituation phase after which they can freely move amidst the anemones. Otherwise anemone-naïve individuals of the same species as those habituated would not be attacked by anemones. This mechanism seems to be very similar for the majority of facultative symbionts studied here, shown with a few examples for several species that all are having the same reactions. In addition the data pooled with respect to state of habituation and type of transfer experiment support this notion at an adequate significance level.

Concerning our experimental approach, it has to be made clear that manipulating decapods by hand might include the risk that substances from previous experiments could be transferred from one crab to another. But in this case „contaminated“ anemone-naïve crabs should not be stung by anemones. Our results do not give any indication of such mistakes, and therefore show that our method of handling decapods can be considered valid. Another point to be made is that anemone-naïve decapods might provoke attacks by anemones when they move fitfully in order to escape. However, anemones attacked both moving and not moving test crabs.

How do our results compare with the experiments made by CALADO *et al.* (2007), showing that several species collected from anemones prefer other locations when they have the choice? In our *Anemonia* meadow this might be a matter of territoriality, as the infralittoral outside the meadow is inhabited by virtually the same species. Thus finding a territory for some individuals might be linked to acquiring anemone adaptation, since other putative territories are occupied. But once the decapods are adapted, they gain the benefit of anemone protection. In aquarium experiments, as have been made by CALADO *et al.* (2007), this effect should remain undetected, as a single test decapod per se has no territory fights.

The indication of population or even species specificity of the habituation in Saline Bay, rather than the habituation to a single anemone individual, is also of peculiar interest. In principle, a decapod could be habituated to a single anemone individual, a clone of genetically identical individuals, a population or even a species. At the moment it is not clear if the habituation is made by some kind of chemical camouflage by mucus transfer from the anemones as in clown fish (SCHLICHTER, 1976; GIESE *et al.*, 1996; see, however MIYAGAWA & HIDAKA, 1980) or if the decapod actively produces protecting substances (CRAWFORD, 1992). In the latter case one could expect some pre-adaptation in anemone-naïve decapods, which is not supported by our experiments. These rather support the idea of a protection acquired in contact with the anemones, thus being a genuine habituation. Future experiments should therefore check this in more detail, e.g. by comparing decapods being adapted to other anemone species with those adapted to *Anemonia viridis*. In particular the genetic composition of the anemone population should be evaluated, as the presence of one or only a few clones in our meadow and/or in Saline Bay may hide some gradual adaptation that becomes obvious only after testing anemones in a larger area.

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## SUMMARY

### Field experiments on the association of decapod crustaceans with sea anemones, *Anemonia viridis* (Forsskal, 1775)

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During numerous night snorkel trips we found 15 decapod species as facultative symbionts in an *Anemonia viridis* meadow in Saline Bay (Adriatic Sea). Removal and introduction field experiments with both symbiotic (taken from anemones) and anemone-naïve decapods (taken from somewhere else) showed that decapods associated with one anemone individual were also not attacked or stung by other anemones within the same meadow and the near neighbourhood. Naïve decapod individuals were attacked, no matter what species they belonged to, or whether other individuals of these species were found in the meadow or not. It is suggested that decapods go through an individual habituation phase before they become symbionts. Once habituated, they are protected from neighbouring anemones as well. The results are discussed with respect to earlier species lists and aquarium experiments. broj 8